Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1032 (2004) 289–297
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Capillary electrophoresis in the determination of anionic catecholamine metabolites from patients' urine

Heli Sirén [a,b,*], Marjo Mielonen [a], Mare Herlevi [a]

[a] *Department of Chemistry, University of Helsinki, P.O. Box 55, FIN-00014 Helsinki, Finland*
[b] *Technical Research Centre of Finland, VTT Processes, P.O. Box 1602, FIN-02044 VTT Espoo, Finland*

Abstract

A micellar electrokinetic capillary chromatographic method for determining anionic catecholamine metabolites from patient urine samples was established. The optimum electrolyte solution (pH 10.6) was made of tetraborate and sodium dodecyl sulphate into water. Furthermore, studies were focused to optimize solid-phase extraction clean-up steps to concentrate patient urine samples for identification of catecholamines with UV detection. The water-micelle distribution coefficients (octanol–water partition coefficient, $\log P_{ow}$) for the analytes were determined by conductometric titration. High plate numbers (120 000–200 000/40 cm detection window) and small diffusion coefficients (2.00–3.50 $\times$ 10^{-6} cm^{-2} s^{-1}) resulted in high resolution and symmetry of the analyte zones. The standard deviations of the migration times and the peak heights were less than 3 and 7%, respectively. The octanol–water coefficients increased in the order of decreased pK_{a1} value of the analytes, why separation between structurally related vanillinic mandelic acids could be obtained. Limits of detection and quantification were around 0.05 and 0.1 μg/ml, respectively, except for dopamine. The concentrations of the catecholamine metabolites in the studied patient urines varied from 0.186 to 76.4 μg/ml. The results showed evidences of serious diseases among the patients.

Keywords: Octanol–water partition coefficients; Capillary electrophoresis; Catecholamines

1. Introduction

Screening of catecholamine metabolites in patient samples is an important part of diagnosis of pheochromocytoma, Parkinson disease and stress. The main analytical problems in determination of the catecholamine metabolites are their similar structures and small concentrations in biological fluids. Catecholamines have important role in the release of fatty acids from adipose tissue and in the excretion of hormones. In addition, catecholamines and their metabolites are involved in variety of regulatory systems [1].

Catecholamines are adrenaline, noradrenaline and dopamine, which are transmitters in the nerve systems. They are synthetised in pheripherial symphatic nerves, core of adrenal gland, and in adrenergic neurons from the amino acid L-tyrosine, which is extracted from intake of food [2].

Metabolism of catecholamines influences the symphatic effect, which mainly stops the metabolism and activates the cell intake [2]. The cell intake is a very efficient mechanism guided by the primary catecholamine, adrenaline. Some of the compounds, which are involved in inside the cell taking are self-diffused or they are transferred to gliacells by the active transport system. Catecholamines are methylated to phase I metabolites by the enzyme, catechol-*O*-methyltransferase (COMT). Their phase II metabolism is caused by monoamine oxidase (MAO). Regardless of the route, the final products are the same: Dopamine is metabolized to homovanillic acid (HVA), noradrenaline and adrenaline form vanillic mandelic acid (VMA) and 4-hydroxy-3-methoxyglycolaldehyde. The by-products are, e.g. 3-methoxytyramine (3-MTA), normetanephrine (NM) and methanephrine (M) [1–3]. All metabolites are secreted to urine [1,4]. The normal concentrations of VMA, HVA and 5-HIAA in Finnish health population are between 1.3 and 7.6 mg/l. The amounts of the other metabolites stay below 0.7 mg/l.

Catecholamines are determined with high-resolution gas chromatography (HRGC), high-performance liquid chromatography (HPLC) and high-performance capillary electrophoresis (HPCE, CE). The HRGC–MS technique gives reliable results, but the analytes must be derivatized to get the catecholamines volatile [5]. HPLC techniques used for catecholamine analyses are based on ion-pair for-

* Corresponding author.
E-mail address: heli.siren@vtt.fi (H. Sirén).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.12.034

mation and reversed-phase separation, but, however, the separation efficiency is not good for extracted samples. Lately, capillary electrophoresis technique has shown to have resolution enough to separate cationic catecholamines and methoxy-catecholamines [5]. The advantage of the CE method over chromatographic separation methods is the need of small volumes of samples and electrolyte solutions combined with the speed of the analysis and high resolution of the sample zones during migration. In addition, mostly no large-scale sample pretreatment is needed. A problem, however, is poor sensitivity of the detectors, which in commercial instruments are UV-Vis [3–5], laser-induced fluorescence (LIF) or electrospray ionization (ESI) MS detectors [6,7]. The performance of electrochemical detector has also been tested in catecholamine analyses [8–11].

The CE techniques applied for their separation have been capillary zone electrophoresis (CZE) [1], micellar electrokinetic capillary chromatography (MECC) [11] and capillary isotachophoresis (CITP) [12]. Catecholamines have been measured in electrolyte solutions containing acetates [4–6,8], phosphates [8,10], borate [8], citrate [8] and organic solutions [8,9]. Especially, anionic catecholamines have been studied in borate–EDTA electrolyte solution [12] to get better separation efficiency with complex formation.

This study was focused on the development of a capillary electrophoresis method for determination of free anionic catecholamine metabolites from urine samples taken from patients having cancer and stress diseases. In addition, studies were focused to clean the samples from urine matrix components and to concentrate the analytes with polymer-based solid-phase extraction (SPE). Electrophoretic mobilities, diffusion coefficients and analyte-micelle partition coefficients were measured under optimized micellar capillary chromatographic medium to find out the solution to separate structure-related metabolites from each other. The research was part of the studies made in developing CE techniques to monitor drugs in clinical samples in on-line mode with capillary electrophoresis [6,11,12].

2. Experimental

2.1. Capillary electrophoresis instrument

An HP3D CE instrument (Agilent Technologies, Walbronn, Germany) equipped with a photodiode array detection (DAD) system (monitoring wavelength at 200 nm, 214 nm, 220 nm and 254 nm) was used for the analyses. The fused silica capillaries (Composite Metal Services, The Chase, Worcestershire, UK) were 38.5–68.5 cm (separation lengths 30–60 cm) × 50 μm i.d. × 365 μm o.d. The applied voltages were +18 or +20 kV. The samples were introduced into the inlet end of the capillary by a pressure of 0.5 psi (1 psi = 6894.76 Pa) for 3–10 s. The temperature of the capillary was maintained at +25 °C with a liquid coolant system. The sample tray was kept at +28 ± 1 °C by air flowing.

2.2. Other instruments used in the study

The deionized water was made with a Milli-Q Academic Instrument (Millipore, Bedford, MA, USA). pH values of the electrolytes were measured with MeterLab PHM220 LAB–pH meter (Radiometer, Copenhagen, Denmark) and a combination electrode (WTW, Weilheim, Germany), which was calibrated with commercial aqueous buffers of pH 4.00, 7.00 and 10.00 (Merck, Darmstadt, Germany). A second system was needed for pH measurement, when high pH values in the elecrolytes were used. Therefore, a Jenway 3030 pH meter (Jenway, Essex, UK) was needed. The electrodes were calibrated with standard solutions of pH 10.00 and 13.00 (Merck).

All sample and electrolyte solutions were mixed with a Vortex Genie 2 (Prolab, Oriola, Finland) or Sartoroeius BP 301 S (Sartorius, Göttingen, Germany) test tube mixers. Also, Utrawave U1750 water bath (Ultrawave, Cardiff, UK) was used for preparation and degassing of the electrolytes, samples and standard solutions. The chemicals were weighted with a Mettler HL52 instrument (Mettler Instrumente, Zurich, Switzerland).

In sample preparation the instrument for SPE was from J.T. Baker (Deventer, The Netherlands). Sorbents were from IST isolution (IST 101 mixed mode, Internal Sobent Technology, Mid Glamorgan, UK) and from Waters (Oasis HLB, Waters, Taunton, MA, USA). After SPE treatment Thermo IEC Micromax centrifuge (IEC, USA) was use to separate the possible precipitation.

A Radiometer model CDM 3 conductometer connected with a Radiometer PP1042 platinum cell (Radiometer) was performed in determination of critical micelle concentration for calculations of octanol–water partition coefficients ($\log P_{ow}$).

2.3. Materials

All reagents were of analytical grade. DL-4-Hydroxy-3-methoxymandelic acid (vanillic mandelic acid, VMA), DL-3-hydroxy-4-metoxymandelic acid (isovanillic mandelic acid, V′MA), 4-hydroxy-3-methoxyacetic acid (homovanillic acid, HVA), 3,4-dihydroxyphenylalanine (DOPA), 5-hydroxy-3-indoleacetic acid (5-HIAA), trichloromethiazide (TCMA, M_r 380.7 g/mol, pK_a 8.6 [13]), sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$), 3-(cyclohexylamino)-1-propanesulphonic acid (CAPS) and sodium dodecylsulphate (SDS) used in the preparation of electrolyte solutions, timepidium bromide (the micelle marker), acetic acid and ammonia were from Sigma–Aldrich (Steinheim, Germany). Sodium hydroxide solution was from J.T. Baker and methanol from Riedel-de Haën (Seelze, Germany).

2.4. Standard solutions

The 1000 μg/ml stock solutions of the analytes 5-HIAA, HVA, VMA and V′MA were prepared in water, except the

solution made of DOPA which was made into 10% (v/v) acetic acid in water. The working solutions of mixtures were prepared by diluting with water to concentrations needed. TCMA, which was used as the internal standard, was made to 1000 μg/ml solution and used in the samples at 20 μg/ml concentration.

2.5. Urine samples

The patient samples were obtained from the University Hospital of Helsinki (Helsinki, Finland). In storage the samples were kept at −20 and −80 °C. Before sample pretreatment the patient urine samples were either centrifuged to exclude the solid material or filtered through 0.45 μm PTFE membranes (Millipore, Molsheim, France).

For capillary electrophoresis calibration all the 80 samples used in this study were first analysed by screening technique after sample pretreatment with CE. In screening the catecholamine analyte concentrations were estimated with two to three concentration standards. When the peak resolution was not satisfactory (happens, e.g. in samples with high ionic strength) or the ion zones were distorted, the samples were diluted 1:2 (v/v) with the electrolyte solution.

CE quantification was performed with standard mixtures containing the anionic catecholamine metabolites as a mixture at their linear concentration levels (0.1–100 and 0.1–200 μg/ml for 5-HIAA) by using the peak heights of the analytes. The samples were analyzed with four replicate injections of each sample.

2.6. Sample preparation

SPE was used for cleaning and concentrating analytes in patient samples. In our earlier studies with cationic catecholamines we have used Oasis HLB copolymer SPE sorbents, which gave good recoveries for catonic catcholamines dopamine, 3-metoxytyramine, DL-normetanephrine and DL-metanephrine [15,16]. Therefore, in this study polymer-based Oasis and IST 101 mixed mode (International Sorbent Technology) sorbents were used for pretreatment of anionic catecholamines (vanillic mandelic acid, isovanillic mandelic acid, DOPA, 5-HIAA and HVA). We have also tested silica-based reversed-phase material C_{18}, but according to our calculations the recoveries of the analytes were not satisfactory for quantification of small concentrations.

Secondly, the reason to use polymer sorbent was the fast cleaning and high capacity of the materials for the anionic catecholamines. The Oasis SPE materials were conditioned with 1 ml of both methanol and water at that order. Urine (1 ml) was applied onto the conditioned sorbent. Matrix compounds were washed with methanol–water (5:95, v/v) solution to waste and the analytes were eluted from the sorbent with methanol.

The eluates obtained from SPE were evaporated under nitrogen at 40–50 °C. The precipitation was dissolved into 200 μl of methanol–sodium hydroxide–electrolyte (tetraborate–SDS) mixture (40:40:20, v/v/v). Methanol was used to enhance the solubility of the concentrate. The samples were centrifuged, after which 100 μl of the final solution was mixed with 20 μl of 100 mg/l stock solution of TCMA (internal standard, ISTD), which was used as the marker to monitor the repeatability of the CE separation.

The blank urine (pooled from urines from health persons) was used for determination of the limits of quantification for each analyte. It was purified from catecholamines with SPE cleaning. It was the urine marix solution flowing through the sorbent, when the catecholamines were adsorbed onto the sorbent.

2.7. Electrolyte solutions

The anionic catecholamine metabolites were first separated with CZE in basic electrolyte solutions made of sodium tetraborate, ammonium acetate and CAPS at pH and concentration ranges of 8.9–11.5 and 20–70 mM, respectively. However, in CZE the resolution was not good enough between HVA and DOPA or V′MA and VMA. Therefore, a MECC system was developed and it was based on the use of the most stabile performance of sodium tetraborate solution.

In MECC tests, to optimize the concentrations of the chemicals and the pH in the tetraborate solution, different amounts of the buffering (10–70 mM) and micelle forming (10–100 mM) chemicals were used, first keeping the experimental conditions at pH 10.6. When the pH was adjusted, six solutions containing 25 mM sodium tetraborate and 65 mM SDS were prepared with pH to 9.82, 10.07, 10.32, 10.82 and 11.07. The anionic catecholamine metabolites were finally separated in an electrolyte solution containing 25 mM sodium tetraborate–25 mM SDS solution (pH 10.82 adjusted with 0.1 M NaOH). However, the patient urine samples were determined by replacing 25 mM with 65 mM SDS to get better resolution between HVA, DOPA and some marix compounds. The electrolytes were filtered with 0.45 μm nylon membranes and degassed with ultrasonication for 15 min before use.

2.8. Capillary conditioning

The new silica capillaries were conditioned by pressure flushing with 20 psi by using 0.1 M sodium hydroxide and water for 10 min each. After that conditioning was continued with the electrolyte solution for 30 min. Between analyses the capillary was flushed with the electrolyte solution for 2 min.

2.9. Procedures

The analytical parameters discussed below were determined by CE–UV using five replicate analyses of each standard solution and the samples, and the results given are mean values obtained. The neutral electroosmotic flow

Table 1
Physicochemical properties of anionic catecholamine metabolites

	5-HIAA	HVA	DOPA	VMA	V′MA
M_r (g/mol)	191.20	182.19	197.19	198.17	198.17
pK_{a1}[a]	4.51	4.43	2.28	2.25	3.05
pK_{a2}[a]	15.59		12.49	16.89	18.13
pK_{a3}[a]	9.92	7.85	9.56	9.94	9.72

[a] Values calculated with Pallas 1.2 program.

(EOF) marker used was methanol. Migration marker for micelles was timepidium bromide (M_r 400.40 g/mol).

2.10. Calculation of pK_a values

The pK_a values for catecholamines in aqueous conditions were predicted using Pallas 1.2 program (CompuDrug Chemistry, Budapest, Hungary) and they are presented in Table 1.

2.11. Measurements of LODs, LOQs and linearity

The limits of detection (LODs) of the anionic catecholamine metabolites were determined in the optimized micelle electrolyte solution with signal-to-noise ratio (S/N) of 3. The limits of quantification (LOQs) for the analytes in SPE-purified urine were calculated at S/N 3. Correlation of the analyte concentrations from 0.1 to 100 μg/ml using peak heights were measured in both water and in SPE-purified urine. The exception was 5-HIAA, for which a range of 0.1–200 μg/ml was needed due to the high concentrations in some real samples.

2.12. Determination octanol–water partition coefficients

The octanol–water partition coefficients ($\log P_{ow}$) for the anionic catecholamines were determined by conductometric titrations by using electrolyte solution both with and without 30 mM SDS, which was the highest concentration possible to be performed in the SDS solvent additions. In addition, only with the chemical additions differences in the conductance of the electrolyte could be recognized. The titration was made with 50 and 100 μl solvent volume additions. The critical micelle concentration, CMC, was 2.9 mM and it could be calculated from the conductometric titration results. The octanol–water partition coefficients for the analytes were calculated by using the four equations:

$$P = \frac{kV_{aq}}{V_{mc}} \quad (1)$$

$$k = \frac{t_r - t_0}{t_0(1 - t_r/t_{mc})} \quad (2)$$

$$\rho = \frac{M}{N_A V} = \frac{m}{V} \quad (3)$$

$$V_{mc} = V_{tot} - V_{free} \quad (4)$$

where P is the partition coefficient, k the partition factor of the analyte corresponding to retention factor in chromatography, V_{aq} volume of aqueous phase, V_{mc} the volume of micelle phase, t_r migration time of the analyte, t_0 migration time of electroosmosis, t_{mc} migration time of marker for micelles (timepidium bromide, M_r 400.40 g/mol), M_r molar mass, N_A Avogadron number, ρ density and m mass. V is $V_{aq} + V_{mc}$, V_{tot} the total volume of the surfactant, V_{free} the volume of monomeric surfactant (non micellar), which can be estimated to be the same as the critical micellar concentration (CMC). The total micellar volume was calculated from the volumes of the groups in the surfactant [14].

The electrophoretic mobilities of the anionic catecholamines were calculated by using equations: $\mu_{tot} = \mu_{eo} + \mu_{ep}$ and $\mu = L_{tot}L_{det}/Vt_r$. In this case V is applied voltage, L_{tot} total length of the capillary, L_{det} length of the capillary to detection, μ_{tot} total mobility of the compound, μ_{eo} the electrophoretic mobility and μ_{ep} is the apparent electrophoretic mobility of an analyte.

3. Results and discussion

The capillary electrophoresis (CE) separation of the anionic catecholamine metabolites was optimized with adjusting the chemical parameters in the electrolyte solution and optimizing the instrumental values on the basis of the electrolyte composition and its pH (ionic strength of the solution). The other criteria were reproducibility of the separation, the analysis time and good resolution of two isomeric catecholamine metabolites, VMA and V′MA. The final MECC analysis could be completed within 20 min under 20 kV voltage in 25 mM sodium tetraborate–25 mM SDS solution (pH 10.82). The analysis was rapid enough for determination of patient urine samples with medium-throughput technique (Fig. 1A and B). Repeatability of the migration times in CE were also good (R.S.D. 3%) and therefore the urine samples could be analyzed and quantified with the method optimized. More selective monitoring of HVA, VMA and V′MA would be obtained at 254 nm, if the concentrations were higher. However, the sample could not be more concentrated without lacking of separation efficiency.

Accuracy of the CE technique was tested with two reference mixtures containing all the analytes in moderately low concentrations. The mixtures were analyzed with the methods optimized for the study. The relationship of peak height in the electropherogram with the concentration was measured by using linear calibration made with peak height as a function of the analyte concentration. The results showed at the range of 1–10 μg/ml correlation higher than 0.99 for the analytes.

The repeatability of the method was optimized with standard mixture and one urine sample containing the identified analytes 5-HIAA, TCMA (ISTD, added to the sample), HVA, VMA and V′MA. Migration times of the analytes in the real samples using +18 kV voltage were 9.10 min

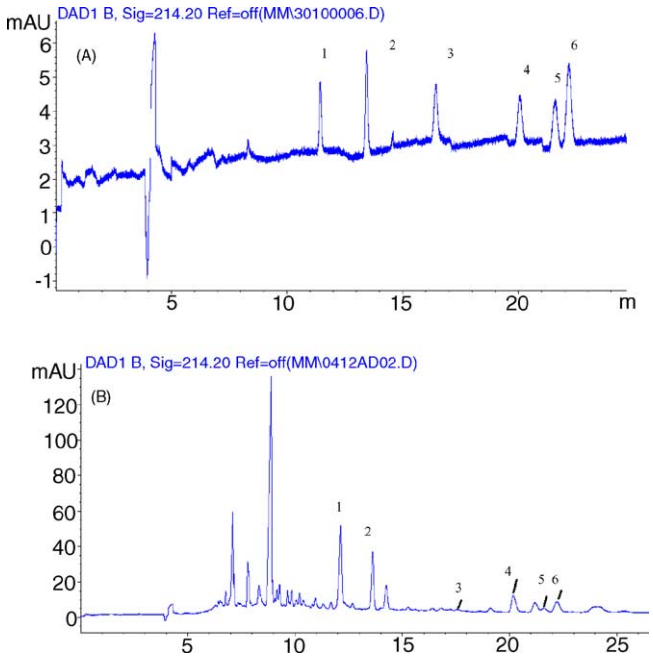

Fig. 1. Electropherogram of anionic catecholamine metabolite separation: (A) standard mixture and (B) patient urine sample after SPE cleaning. Peaks in (A): (1) 5-HIAA, 10 μg/ml; (2) TCMA (ISTD); (3) DOPA, 20 μg/ml; (4) HVA, 10 μg/ml; (5) VMA, 10 μg/ml; (6) V′MA, 10 μg/ml. Peaks in (B): (1) 5-HIAA165 μg/ml; (2) DOPA 2.71 μg/ml; (3) HVA 40.8 μg/ml. Electrolyte solution: 25 mM sodium tetraborate–65 mM SDS (pH 10.82), current 98 μA, voltage +18 kV, injection with 0.5 psi pressure for 3 min, detection at 214 nm (UV), capillary 40/48.5 cm (L_{det}/L_{tot}).

(R.S.D. 2%), 10.32 min (R.S.D. 7%), 16.76 min (R.S.D. 9%), 22.22 min (R.S.D. 6%) and 22.73 min (R.S.D. 7%) for the analytes as their migration order. The standard deviations of the peak hights were 9, 21, 22, 34 and 46% for 5-HIAA, TCMA, DOPA, VMA and V'MA in spiked blank urine (see Section 2.6), respectively. However, the MECC method was more repeatable with standards. Migration times using +20 kV voltage for 5-HIAA, TCMA, HVA, VMA and V′MA were 6.33 min ± 0.01 (R.S.D. 0.08%), 7.62 min ± 0.01 (R.S.D. 0.03%), 8.16 min ± 0.05 (R.S.D. 0.57%), 8.47 min±0.05 (R.S.D. 0.58%) and 8.92 min±0.35 (R.S.D. 1.87%), respectively.

It was observed that the higher the migration time, the lower was the R.S.D. value of the peak area. The reason for this was the background, which was low in the case of high concentrations. However, due to the partly dissociation of DOPA in the electrolyte solution at pH 10.82, the standard deviation of its migration increased. The reproducibility of peak areas of 5-HIAA, TCMA, HVA, VMA and V′MA were 0.9944, 0.9959, 0.9847, 0.9998 and 0.9995 for standards and 0.5737, 0.9581, 0.9927, 0.9995 and 0.6968 for the analytes in real samples, respectively. However, better correlation was achieved when peak heights were used: 0.9941, 0.9941, 0.9889, 0.9951 and 0.9821 for standards and 0.9481, 0.9843, 0.9760, 0.7489 and 0.9782 for the analytes in patient samples, respectively. Therefore, peak heights were used in determinations of patient urine samples.

3.1. Limits of detection and quantification

The LODs of the catecholamines were determined in aqueous electrolyte at signal to noise ratio (S/N) of 3. As ca. 6 nl injection volume was used in the analyses, the limits of detection from 0.6 to 3.0 μg/ml correspond to 3 to 14 fmol of different analytes. The LOQs were measure using SPE-purified urine as the matrix (free from catecholamines), which was spiked with the analytes (see Section 2.6). The solutions were pretreated as samples and the concentrations were measured with MECC optimised for urine samples (electrolyte containing 65 mM SDS). The LOQs for 5-HIAA, DOPA, HVA, VMA and V′MA were 1.26 mg/l (6.8 μM), 0.672 mg/l (1.8 μM), 3.16 mg/l (16.2 μM), 1.67 mg/l (8.6 μM) and 1.71 mg/l (5.0 μM) with signal-to-noise ratios (S/N) of 7.9, 14.9, 6.32, 5.99, 5.85, respectively.

Concentrations of all the analytes were from 1 to 10 mg/l. The results showed that the concentration correlations (R^2) to peak hights were 0.9481, 0.9843, 0.9760, 0.7489 and 0.9782 for 5-HIAA, DOPA, HVA, VMA and V′MA, respectively.

3.2. Diffusion coefficients and plate numbers

Diffusion coefficients were calculated for 5-HIAA, HVA, VMA and V′MA in the 25 mM tetraborate–65 mM SDS electrolyte solution, which pH was adjusted to 10.82 with 0.1 M NaOH. The results (Fig. 2) were obtained with a 10 μg/ml standard mixture. The electrophoretic mobilities were calculated with $\mu_{tot} = \mu_{eo} + \mu_{ep}$ and $\mu = L_{tot}L_{det}/Vt_r$ as described in Section 2.12. Diffusion coefficients were calculated from $D = (\mu_{eo} + \mu_{ep})VL_{det}/2L_{tot}N$, where the plate number $N = 16\,(t_r/w_r)^2$. Diffusion can also be measured with the Einsein equation $D = \sigma_D^2/2\,\Delta T$, where the diffusion variance (σ_D^2) is the increased due to longitudial diffusion. The calculated results from the electrophoretic separations are given in Figs. 3 and 4. In the real samples it was noticed that the urine matrix compounds (evaluated from SPE-treated urine samples) were disturbing the separation of VMA and V′MA, why their diffusion coeffiecients were measured to be equal. Fig. 2 shows that the diffusion coefficients, i.e. diffusion variances correlate highly with the analyte retentions in a second-order function, as expected according to the Einstein equation.

The plate numbers are inversely proportional to diffusion ($N = \mu_{ep}V/2D$), why the figures are in inverse relation to one another (Fig. 3). The plate numbers (N) of the analytes were between 120 000 and 200 000 (capillary with 40 cm detection window). They were high when the electrophoretic mobility is slow. Furthermore, VMA and V′MA have same molar masses and very similar structures and they could only be separated due to partitioning into the

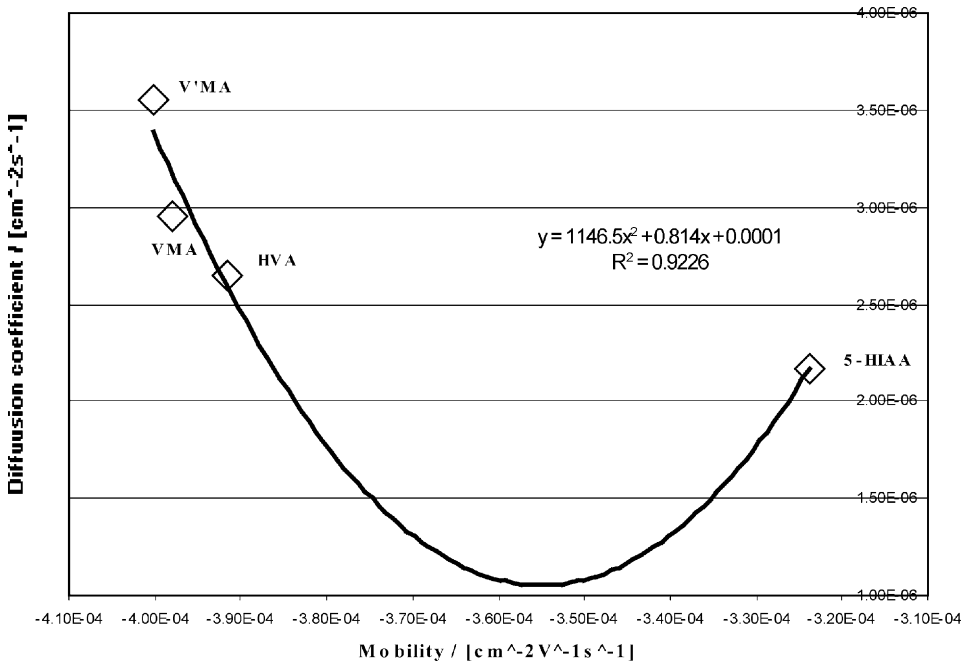

Fig. 2. Diffusion coefficients of the catecholamines versus electrophoretic mobilities. Experimental conditions as in Fig. 1.

micelle. However, the high N values did not correlate to better separation efficiency in the case of VMA and V′MA. In this optimized MECC system the resolution between VMA and V′MA was 2.5.

3.3. Octanol–water partition coefficients

Often the logarithms of the octanol–water partition coefficients ($\log P_{ow}$) of analytes have been used to describe the retention of the analytes in MECC. In general, the relationship between the values in different micellar electrolyte solutions have been studied by a number groups and in many cases the correlations have been extremely good [17]. However, in MECC electrolyte conditions there are other interactions, too, why the values of analyte retention factors (k) can be used to correlate P_{ow}. Fig. 4 and Table 2 show the octanol–water partition coefficients of anionic catecholamine metabolites. From our results it can be concluded

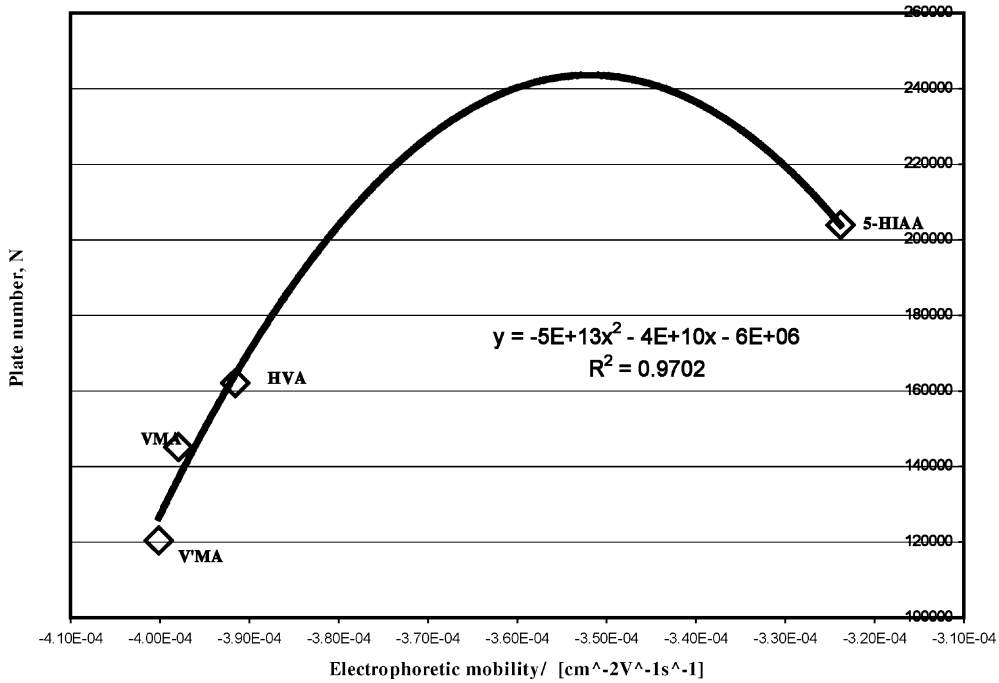

Fig. 3. Plate numbers of the catecholamines versus electrophoretic mobilities. Experimental conditions as in Fig. 1.

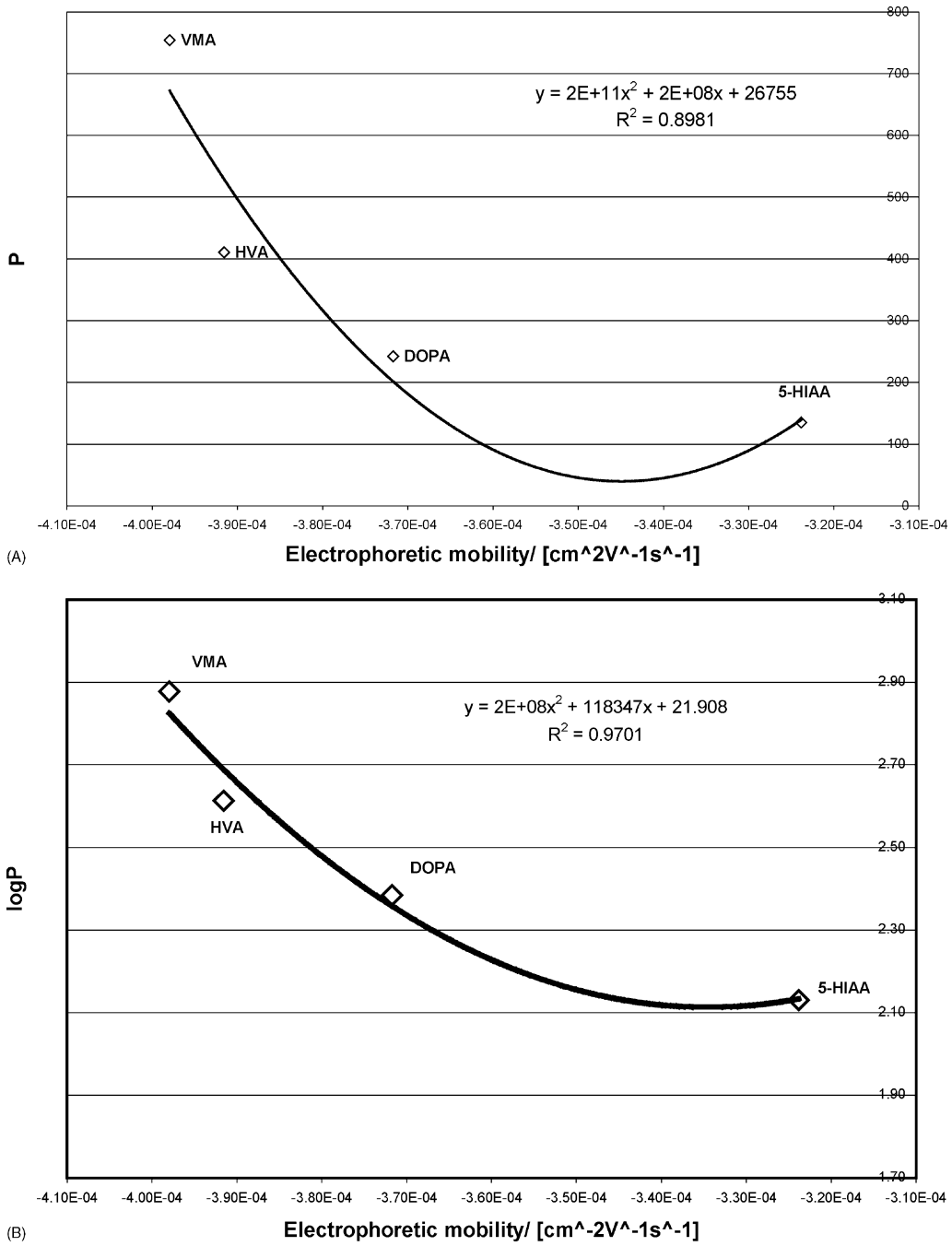

Fig. 4. Octanol–water partition coefficients of the catecholamines versus electrophoretic mobilities.

that the $\log P_{ow}$ and P-values of the analytes correlate highly with the analyte retention in a second-order function. The reason not to get a linear relationship between the parameters (Eq. (1)), was the possible complex formation of tetraborate with the analytes, which affected the most in 5-HIAA retention. The figure also shows that anionic catecholamines containing two hydroxyl groups are clustered with their values in the same area, but 5-HIAA having only one OH group had clearly different P_{ow}. In the literature no values of $\log P_{ow}$ for the anionic catecholamine metabolites were found.

3.4. Anionic catecholamines in samples

The anionic catecholamines in urine samples were identified with spiking the sample (200 μl sample vessels) with 5 μM of the catecholamine mixture. It was noticed that the concentrations of the anionic catecholamines were on the average between 1.3 and 7.6 μg/ml. However, there were nine (six extremely high) samples high in 5-HIAA (Fig. 5A), two samples high in V′MA and VMA (Fig. 5B) and four samples high in HVA (Fig. 5C), but only two samples exceeded the average amount of DOPA (Fig. 5D). Especially,

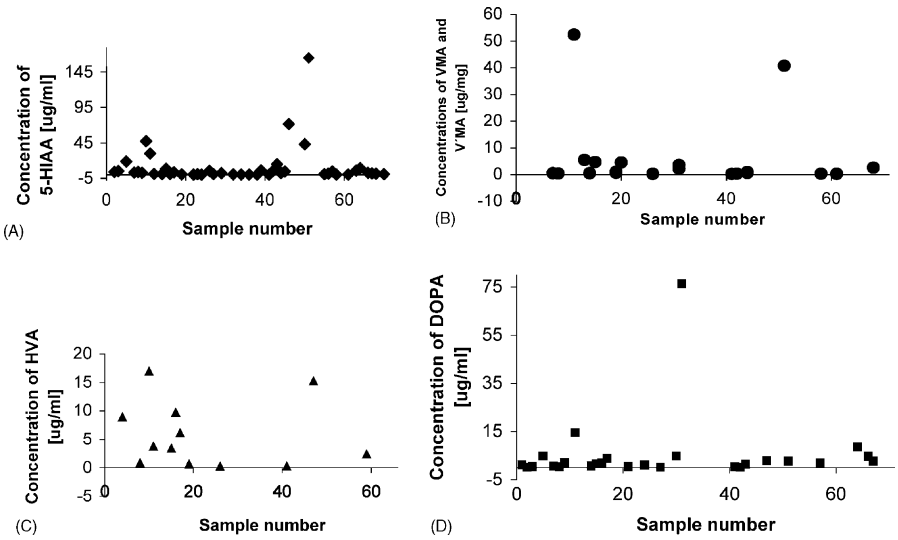

Fig. 5. Results of the patient urine samples: (A) DOPA; (B) VMA and V′MA; (C) 5-HIAA; (D) HVA. Experimental conditions as in Fig. 1.

5-HIAA is the metabolite of serotonine. The existence of it can be used to diagnosis of tumours in intestines. The amount of 5-HIAA is between 1.3 and 7.6 mg/l and concentrations of the other anionic catecholamine metabolites stay below 0.7 mg/l in normal person urine. According to the results obtained in our study, the concentrations of the catecholamine metabolites in the patient urines varied from 0.186 to 76.4 μg/ml. Therefore it may be concluded that the urine samples may show possible evidences of serious diseases among the patients.

3.5. CE results versus HPLC

Usually, catecholamines have been analysed with ion exchange (IEC) and reversed-phase liquid chromatography (RP-HPLC) [18–20]. Several publications have compared the use of HPLC and CE as separation techniques with UV detection in determination of cationic catechlamines in urine, plasma and other biological matrices. In general, the results obtained by HPLC and CE correlate well. CE provides two to seven times as fast analysis with high column efficiency, whereas HPLC method provides, at least two to three times better sensitivity due to high-volume injection and sorbent capacity. The sample purification needed for LC analysis, in turn, often leads to cleaner chromatograms and lower limits of quantification [10,21–23]. However, with CE both the cationic and anionic catecholamines can be monitored and quantified with high reliability due to the high resolution and the possibility to use fast, two-partial analysis: cationic catecholamines and methoxy-catecholamine metabolites in acid electrolyte and anionic metabolites in micellar basic electrolyte, as described in this study.

Table 2
Parameters measured with a standard mixture under conditions used in urine sample determination (see Section 2)

Analyte	t_r (min)	k	P_{ow}	$\log P_{ow}$	μ_{ep} (10^{-4} cm^2 V^{-1} s^{-1})
5-HIAA	9.1	1.8	135.2	2.1	−3.2
TCMA	10.3	2.3	170.1	2.2	−3.5
DOPA	12.5	3.2	242.2	2.4	−3.7
HVA	16.8	5.5	410.6	2.6	−3.9
VMA	22.9	10.0	754.0	2.9	−4.0
EOF	3.73				4.81
Micelle	47.23				

The electrolyte solution in which the measurements were performed contained 25 mM sodium tetraborate–30 mM SDS, pH 10.82.

4. Conclusions

The developed tetraborate–SDS MECC technique was useful for separation and determination of anionic catecholamine metabolites in urine samples. Baseline separation was obtained for 5-HIAA, TCMA (ISTD), DOPA, HVA, VMA and V′MA. The identification of DOPA was difficult, especially, when the analyte was at low concentration. Dopamine (pK_{a3} 12.49) was not fully dissociated at 10.82, which was the pH of the electrolyte. The analysis time was less than 30 min.

Different physical parameters, like electrophoretic mobilities, diffusion coefficients and octanol–water distribution coefficients for the analytes were also measured in the tetraborate–SDS electrolyte solution.

The patient urine samples contained different amounts of the metabolites. However, same really high concentrations of them were calculated from the analysis data, which correlate to a disease. The values obtained here were on the average slightly above the upper limits of the concentrations of a healthy person.

Acknowledgements

Financial support of the Academy of Finland is gratefully acknowledged (H. Sirén, project number 43326). The

authors thank Dr. Ulla Karjalainen for the patient urine samples and Dr. Johanna Suomi for helping with the $\log P_{ow}$ measurements.

References

[1] J. Bergquist, A. Sciubisz, A. Kaczor, J. Silberring, J. Neurosci. Methods 113 (2002).
[2] Koulu-Tuomisto, Pharmacology and Toxicology, Medicina, Gummerus, Jyväskylä Finland, sixth ed., 2001 (in Finnish).
[3] H. Sirén, U. Karjalainen, J. Chromatogr. A 853 (1999) 527.
[4] R.P.H. Nikolajsen, Å.M. Hansen, Anal. Chim. Acta 449 (2001) 1.
[5] Z.D. Peterson, D.C. Collins, C.R. Bowerbank, M.L. Lee, S.W. Gravers, J. Chromatogr. B 776 (2002) 221.
[6] K. Vuorensola, Ph.D. thesis, University of Helsinki, Helsinki, 2002.
[7] D.-C. Chen, D.-Z. Zhan, C.-W. Cheng, A.-C. Liu, C.-H. Chen, J. Chromatogr. B 750 (2001) 33.
[8] C. Radja, K. Bencsik, L. Vécsei, J. Bergquist, J. Neuroimmunol. 124 (2002) 93.
[9] X. Li, W. Jin, Q. Weng, Anal. Chim. Acta 461 (1) (2002) 123.
[10] K. Vuorensola, H. Sirén, U. Karjalainen, J. Chromatogr. B 788 (2003) 277.
[11] J. Caslavska, E. Gassmann, W. Thormann, J. Chromatogr. A 709 (1995) 147.
[12] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2002) 1242.
[13] M.-L. Riekkola, J.H. Jumppanen, J. Chromatogr. A 735 (1996) 151.
[14] J. Suomi, S.K. Wiedmer, M. Jussila, M.-L. Riekkola, J. Chromatogr. A 970 (2002) 287.
[15] K. Vuorensola, H. Sirén, J. Chromatogr. A 895 (2000) 317.
[16] K. Vuorensola, H. Sirén, U. Karjalainen, J. Chromatogr. B 788 (2003) 277.
[17] S. Wiedmer, M.-L. Riekkola, Rev. Anal. Chem. 18 (1–2) (1999) 67.
[18] M. Radjairpour, H. Raster, H.M. Liebich, Eur. J. Clin. Chem. Clin. Biochem. 32 (1994) 609.
[19] M. Hay, P. Mormède, J. Chromatogr. B 703 (1977) 15.
[20] P. Volin, J. Chromatogr. B 655 (1994) 121.
[21] T. Fujii, S. Kawabe, T. Hoike, T. Taguchi, M. Ogata, J. Chromatogr. B 730 (1999) 41.
[22] A. West, M. Frost, H. Köhler, Int. J. Legal Med. 110 (1997) 251.
[23] C. Bory, C. Chantin, R. Boulieur, J. Chromatogr. 578 (1992) 283.